Reassessment of HLA-G isoform specificity of MEM-G/9 and 4H84 monoclonal antibodies.

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Public Summary:
Human leukocyte antigen G (HLA-G) is a non-classical HLA class I molecule thought to play a key role in maternal-fetal tolerance. The HLA-G primary transcript generates seven alternative mRNAs that encode four membrane-bound proteins (HLA-G1 to HLA-G4) and three soluble proteins (HLA-G5 to HLA-G7). HLA-G possesses a direct inhibitory effect on the proliferation and the cytolytic function of peripheral blood natural killer cells and cytotoxic T lymphocytes, the maturation and function of dendritic cells, and the alloproliferative responses of $CD4^+$ T cells. Although initial studies suggested that HLA-G expression is restricted to extravillous cytotrophoblasts, expression was subsequently reported in a wide variety of other human tissues and tumor cells. However, consensus as to the validity of these collective findings has proven difficult because the antibodies used to define the temporal and spatial expression patterns of HLA-G remain incompletely characterized. The aim of our study was to examine two of the most widely used commercial monoclonal antibodies (mAbs) against HLA-G (MEM-G/9 and 4H84). To accomplish this, we assessed MEM-G/9 and 4H84 immunoreactivity to both denatured and native HLA-G antigens using flow cytometry, immunofluorescence (on live and fixed cells), and western blotting in a variety of human cell lines whose HLA class I protein expression pattern is well characterized. On the basis of our studies, we discovered that MEM-G/9 recognizes not only full-length HLA-G but also truncated forms and that 4H84 shows non-specificity under certain methodological conditions.

Scientific Abstract:
Human leukocyte antigen (HLA)-G is a non-classical HLA class I molecule thought to play a key role in maternal-fetal tolerance. Although initial studies suggested that HLA-G expression is restricted to extravillous cytotrophoblasts, expression was subsequently reported in a wide variety of other human tissues and tumor cells. However, consensus as to the validity of these collective findings has proven difficult because the antibodies used to define the temporal and spatial expression patterns of HLA-G remain incompletely characterized. The aim of our study was to reassess two of the most widely used HLA-G antibodies (MEM-G/9 and 4H84) in HLA-G-positive (JEG-3 and HLA-G transduced) and -negative (dermal fibroblast, mesenchymal stem cell, K562, and Jurkat) lines using flow cytometry, immunofluorescence, and western blotting. We found that MEM-G/9 recognized HLA-G3 by flow cytometry, indicating that its epitope is present on the alpha1 domain of HLA-G. Although 4H84 preferably recognized unfolded HLA-G-free chains, it showed strong non-specificity under certain methodological conditions.